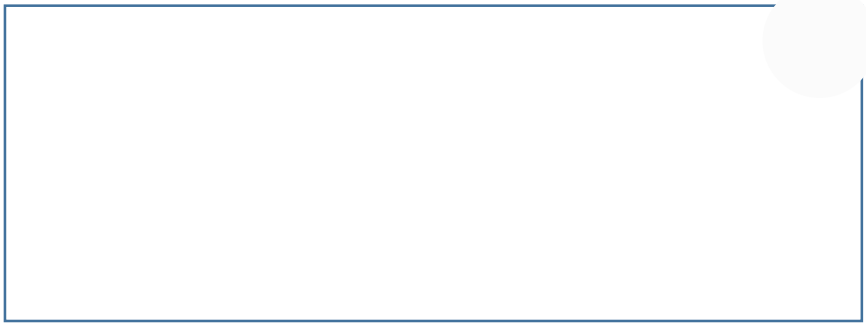
Assembly:



1st Day

EXSP Digestion

Parts	ng/ul	Volume to 2,5 ug (ul)	Buffer x10 (ul)	EcoRI (ul)	XbaI (ul)	SpeI (ul)	PstI (ul)	H_2O to 50ul (ul)

2nd Day

Gel Purification

- See **Protocol from the kit Wizard SV gel and PCR clean up Promega**
- Quantify digestion products

Parts	ng/ul	260/280

Obs: 260/280 in a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

Ligation

Linear Plasmid 50 ng		
Insert : Plasmid		
10x T4 DNA Buffer	2 ul	
T4 DNA ligase 1-5 u	1 ul	
H_20 to 20 ul		

Obs: To determinate the amount of DNA necessary we used the following equation

$$Insert\ ng = plasmid\ ng \times \frac{insert\ bp}{plasmid\ bp} \times insert{:}\,plasmid\ ratio$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium
- Prepare glycerol 40%

3rd Day

Transformation

(see **Transformation Protocol in *Escherichia coli* DH5-α**)

Organism:

Selection:

4th Day

- Inoculate 3 – 4 colonies in a 6 ml LB with the same antibiotic used in the transformation protocol.

5th Day

Miniprep

- Prepare **glycerol stock** of the clones.
- Extract plasmidial DNA (see **Alkaline Lyses Protocol or kit Wizard Plus SV Minipreps Promega or PureLink Invitrogen**)
- Run a preliminary electrophoresis gel.
- Quantify DNA samples.

Assembly Confirmation

- EP Digestion

Assembly	Volume to 300 ng (ul)	Buffer x10 (ul)	EcoRI (ul)	PstI (ul)	H_2O to 10ul (ul)

- Incubate for 2 hours at 37°C.
- Prepare samples for DNA sequencing.
- Run an electrophoresis analysis of the EP digestion

Size expected	Size in gel